

APPENDIX I

CURRENTLY PENDING CLAIMS

22. A fragment complementation system, said system comprising:
a first oligopeptide comprising an N-terminal fragment with a C-terminal break-point, and a second oligopeptide comprising a C-terminal fragment with a N-terminal break-point, wherein said N-terminal fragment and said C-terminal fragment each are derived from a marker protein and reassemble to form a functionally reconstituted marker protein.

23. The fragment complementation system according to Claim 22, wherein said first oligopeptide and said second oligopeptide each further comprise a cysteine residue within 5 amino acid positions of said break-point.

24. The method according to Claim 23; wherein said cysteine residue is at said break-point.

25. A fragment complementation system, said system comprising:
a first oligopeptide comprising an N-terminal fragment fused through a break-point to a flexible polypeptide linker and a first interactor domain; and
a second oligopeptide comprising a second interactor domain and a flexible polypeptide linker fused through a break-point to a C-terminal fragment, wherein said N-terminal fragment and said C-terminal fragment are both derived from a marker protein with a directly selectable signal, and wherein said N-terminal fragment and said C-terminal fragment are obtained according to the method of Claim 1, and wherein said N-terminal and said C-terminal fragment functionally reconstitute said marker protein only upon binding of said first interactor domain with said second interactor domain.

26. The fragment complementation system according to Claim 25, wherein said first and said second oligopeptide further comprise a signal peptide.

27. The fragment complementation system according to Claim 25, wherein said N-terminal and said C-terminal fragments together comprise one of a

contiguous, overlapping or non-continuous sequence of said marker protein and comprise between about 90 to 110% of the total length of said marker protein.

28. The fragment complementation system according to Claim 27, wherein functional reconstitution of said marker protein is enhanced by introducing at least one of the following modifications to at least one of said first and said second oligopeptide sequences:

- i) a randomly-encoded peptide of 3-12 amino acids encoded between said fragment and said flexible polypeptide linker,
- ii) a randomly-encoded peptide of 3-12 amino acids expressed separately and operably fused to the N-terminus of a thioredoxin,
- iii) a cysteine residue encoded between said fragment and said flexible polypeptide linker, or
- iv) 1-3 codon changes per fragment molecule introduced by PCR-amplifying a nucleotide sequence that encodes for said fragment under error-prone conditions to enable more stable folding of a reconstituted marker protein.

29. The fragment complementation system according to Claim 25, wherein said directly selectable signal is a visible phenotypic change or antibiotic resistance.

30. The fragment complementation system according to Claim 25, wherein said protein that has a directly selectable signal is an enzyme.

31. The fragment complementation system according to Claim 28, wherein said first interactor domain is selected from the group consisting of a single chain antibody Fv fragment, an antibody light chain variable region, and a cell surface molecule, and said second interactor domain comprises a randomly encoded peptide inserted into the active site of *E. coli* thioredoxin or a phosphorylation-regulated signal transducer protein.

32. The fragment complementation system according to Claim 31, wherein said cell surface molecule is CD40.

33. The fragment complementation system according to Claim 31, wherein said phosphorylation-regulated signal transducer protein is a tyrosine kinase.

34. The fragment complementation system according to Claim 25, wherein said first interactor domain encodes a polypeptide from a first library and said second interactor domain encodes a polypeptide from a second library.

35. A fragment complementation system, said system comprising:
a first oligopeptide comprising an N-terminal fragment of a β -lactamase fused through a break point to a flexible polypeptide linker and a first interactor domain;
and

a second oligopeptide comprising a second interactor domain and a flexible polypeptide linker fused through a break-point to a C-terminal fragment of a β -lactamase, wherein said N-terminal and said C-terminal fragment functionally reconstitute said β -lactamase upon binding of said first interactor domain with said second interactor domain.

36. The fragment complementation system according to Claim 35, wherein functional reconstitution of said β -lactamase is enhanced by introducing at least one of the following modifications to at least one of said first and said second oligopeptide sequences:

- i) a randomly-encoded peptide of 3-12 amino acids encoded between said fragment and said flexible polypeptide linker,
- ii) a randomly-encoded peptide of 3-12 amino acids expressed separately and operably fused to the N-terminus of a thioredoxin,
- iii) a cysteine residue encoded between said fragment and said flexible polypeptide linker, or
- iv) 1-3 codon changes per fragment molecule introduced by PCR-amplifying a nucleotide sequence that encodes for said fragment under error-prone conditions to enable more stable folding of a reconstituted marker protein.

37. The fragment complementation system according to Claim 35, wherein said randomly encoded peptide of 3-12 amino acids, is a tripeptide, and wherein

a tripeptide fused to said N-terminal fragment is selected from the group consisting of HSE, NGR, GRE and EKR, and a tripeptide fused to said C-terminal fragment is selected from the group consisting of REQ, QGN, DGR GRR and GNS.

38. The fragment complementation system according to Claim 36, wherein said breakpoint of said N-terminal fragment or said C-terminal fragment is within ten residues in either direction from a junction between amino acid residues selected from the group consisting of N52/S53, E63/E64, Q99/N100, P174/N175, E197/L198, K215/V216, A227/G228, and G253/K254.

39. The fragment complementation system according to Claim 36, wherein said break-point of said N-terminal fragment or said C-terminal fragment is within ten residues in either direction of a junction between amino acid residues E197 and L198.

40. The fragment complementation system according to Claim 39, wherein said randomly-encoded peptide of 3-12 amino acids, comprises the tripeptide GRE.

41. The fragment complementation system according to Claim 35, wherein said N-terminal fragment comprises at least one mutation selected from the group consisting of K55E, P62S and M182T.